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Research Article

# Morphology and phylogeny of four new species within Polycephalomycetaceae (Hypocreales) parasitising *Ophiocordyceps* species

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## Abstract

Species of the family Polycephalomycetaceae grow on insects or entomopathogenic fungi and are distributed from tropical to subtropical regions. This study proposed four new species of hyperparasitic fungi from China based on six molecular markers (ITS, SSU, LSU, *TEF-1α*, *RPB1* and *RPB2*) phylogenetic analyses and morphological characteristics. The four new species, i.e. *Pleurocordyceps litangensis*, *Polycephalomyces jinghongensis*, *Po. multiperitheciatae* and *Po. myrmecophilus*, were described and illustrated. *Pl. litangensis*, exhibiting a hyperparasitic lifestyle on *Ophiocordyceps sinensis*, differed from *Pleurocordyceps* other species in producing subulate β-phialides and ovoid or elliptic α-conidia. *Po. jinghongensis* was distinct from *Polycephalomyces* other species, being parasitic on *Ophiocordyceps* sp., as producing oval or long oval-shaped α-conidia and columns of β-conidia. *Po. multiperitheciatae* differed from *Polycephalomyces* other species as having synnemata with fertile head, linear β-conidia and parasitic on *Ophiocordyceps multiperitheciata*. *Po. myrmecophilus* was distinct from *Polycephalomyces* other species, being parasitic on the fungus *Ophiocordyceps acroasca*, as producing round or ovoid α-conidia and elliptical β-conidia without synnemata from the colonies. These four species were clearly distinguished from other species in the family Polycephalomycetaceae by phylogenetic and morphological characteristics. The morphological features were discussed and compared to relevant species in the present paper.

**Key words:** entomogenous fungi, hyperparasite, micromorphology, phylogenetic analyses, taxonomy



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## Introduction

The new family Polycephalomycetaceae was established within clavicipitoid fungi to accommodate *Perennicordyceps*, *Pleurocordyceps* and *Polycephalomyces* based on morphology and phylogenetic analyses (Xiao et al. 2023). The genus *Polycephalomyces* Kobayasi was determined to be a monotypic anamorph genus for the species *Polycephalomyces formosus* Kobayasi (Kobayasi

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1941). In the later taxonomic treatment of this genus, Seifert (1985) accepted four species, i.e. *Po. formosus*, *Po. ramosus* (Peck) Mains, *Po. cylindrosporus* Samson & H.C. Evans and *Po. tomentosus* (Schrader) Seifert. *Polycephalomyces ditmarii* Van Vooren & Audibert has been described as the asexual morph of *Ophiocordyceps ditmarii* (Quél.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (Van Vooren and Audibert 2005). *Paecilomyces sinensis* C.T. Chen, S.R. Xiao & Z.Y. Shi was recombined into *Polycephalomyces sinensis* (Q.T. Chen, S.R. Xiao & Z.Y. Shi) W.J. Wang, X.L. Wang, Y. Li, S.R. Xiao & Y.J. Yao (Wang et al. 2012). The taxon has had a long history of being recognised as incertae sedis in Hypocreales (Kepler et al. 2013; Matočec et al. 2014). Matočec et al. (2014) established the genus *Perennicordyceps* and separated it from *Polycephalomyces* to accommodate *Perennicordyceps cuboidea*, *Pe. paracuboidea*, *Pe. prolifica* and *Pe. ryogamiensis*. *Perennicordyceps* was characterised by acremonium-like and hirsutella-like asexual morphs and perithecia (Xiao et al. 2023). *Pleurocordyceps* was established by combining the species originally assigned to the *Polycephalomyces*. *Pl. sinensis* was designated as the type species of the genus *Pleurocordyceps* (Wang et al. 2021).

Species of Polycephalomycetaceae grow on insects or other fungi, particularly *Ophiocordyceps* species and are distributed from tropical to subtropical regions (Bischof et al. 2003; Wang et al. 2012, 2015a, b; Matočec et al. 2014; Crous et al. 2017; Xiao et al. 2018; Poinar and Vega 2020). Several species of Polycephalomycetaceae have also been reported as hyperparasitic fungi, involving species of *Cordyceps*, *Elaphomyces*, *Hirsutella*, *Myxomycetes* and *Ophiocordyceps* (Seifert 1985; Bischof et al. 2003; Wang et al. 2012, 2015a, b).

South-western China is an area of high fungal biodiversity (Hyde et al. 2018). The rich biodiversity uncovered suggested that further collections could result in the discovery of numerous new taxa (Hyde et al. 2020a, b). In this study, the four novel species presented herein were collected from Yunnan Province and Sichuan Province in China. Morphological observations and phylogenetic analyses showed that these four species were novel and distinct from all other previously-described species in the family Polycephalomycetaceae. The four new species were discovered to be hyperparasites of *Ophiocordyceps* species. *Pl. litangensis*, *Po. jinghongensis*, *Po. multiperitheciatae* and *Po. myrmecophilus* were hyperparasitic on *O. sinensis*, *Ophiocordyceps* sp., *O. multiperitheciata* and *O. acroasca*, respectively. At present, relatively little is known about the mechanisms responsible for hyperparasitism in species of the family Polycephalomycetaceae and our findings provide ideal material for these studies. These findings have expanded the diversity of fungal species in the family Polycephalomycetaceae, providing taxonomic data to support species resource conservation and rational exploitation and utilisation of resources.

## Materials and methods

### Specimens and isolates

Fungal specimens parasitising *Ophiocordyceps* sp. were collected from different regions of south-western China, including Sichuan Province (Litang County) and Yunnan Province (Jinghong City, Yuanyang County, Pu'er City). The specimens were found in moist soils. Geographic information (longitude, latitude

and altitude) of collection were recorded in the field, then specimens were collected in sterilised plastic containers and transported to the laboratory. The micro-morphological characters (Synnemata) were examined using an Olympus SZ61 stereomicroscope (Olympus Corporation, Tokyo, Japan). To obtain axenic culture, the stromata was divided into 2–4 segments with sterilised blades. Each segment was immersed in hydrogen peroxide 30% ($H_2O_2$) for 5 min and then rinsed five times in sterile water. After drying on sterilised filter paper, these segments were inoculated on Potato Dextrose Agar (PDA) plates. The conidial masses at the apex of the stipes were picked with an inoculating needle and immersed in 5 ml of sterilised water for blending. The homogenates were then spread on PDA plates containing 0.1 g/l streptomycin and 0.05 g/l tetracycline. The plates were maintained in a culture room at 25 °C. After purification, the cultures were stored at 4 °C (Wang et al. 2015a). Dry specimens were deposited in the Yunnan Herbal Herbarium (YHH) of Yunnan University. The cultures were stored in Yunnan Fungal Culture Collection (YFCC) of Yunnan University.

### Morphological studies

Cultures on potato extract agar (PDA) were incubated for 21 days at 25 °C and photographed using a Canon 750 D camera (Canon Inc., Tokyo, Japan). For asexual morphological descriptions, microscope slide cultures were prepared by placing a small amount of mycelium on 5 mm diameter PDA medium blocks that were overlaid by a cover slip (Wang et al. 2015a; Tang et al. 2023b). The observations, measurements and photographs of the phialides and conidia were made using a light microscope (Olympus BX53).

### DNA extraction, PCR and sequencing

DNA templates were obtained from cultures using the CTAB method, following that described in Liu et al. (2001). The polymerase chain reaction (PCR) was used to amplify genetic markers using the following primer pairs: ITS4/ITS5 for ITS (internal transcribed spacer gene region) (White et al. 1990), NS1/NS4 for SSU (small subunit ribosomal RNA gene region) (White et al. 1990), LR0R/LR5 for LSU (large subunit rRNA gene region) (Hopple 1994) 2218R/983F for *TEF-1α* (translation elongation factor 1-alpha gene region) (Rehner and Buckley 2005), CRPB1/RPB1Croph for *RPB1* (RNA polymerase II largest subunit gene region) (Castlebury et al. 2004; Araújo et al. 2018), fRPB2-7cR/fRPB2-5F for *RPB2* (RNA polymerase II second largest subunit) (Liu et al. 1999). A total of 25 μl PCR matrix contained PCR 2.5 μl Buffer (Transgen Biotech, Beijing, China), 17.25 μl sterile water, 4 μl dNTP, 1 μl each forward and reverse primer, 0.25 μl Taq DNA polymerase (Transgen Biotech, Beijing, China) and 1 μl DNA template. The matrix and reactions conditions were prepared and performed according to the methods described in previous studies (Xiao et al. 2023).

### Phylogenetic analysis

In order to construct a phylogeny of the major lineages in the family Polycephalomycetaceae, most of the DNA sequences used in this work were derived from previous phylogenetic studies (Xiao et al. 2023). Phylogenetic analyses were

based on sequences of six molecular markers (ITS, SSU, LSU, *TEF-1α*, *RPB1* and *RPB2*), all of which were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). Then the nucleotide sequences were combined with those generated in our study (Table 1). Sequences were aligned using ClustalX v.2.0 (Larkin et al. 2007), adjusted manually and then concatenated in BioEdit v.7.1.1 (Hall 1999). Poorly-aligned regions were removed and adjusted manually using MEGA6 (v.6.0) (Tamura et al. 2013). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fitting likelihood model for the Maximum likelihood (ML) analyses and the Bayesian inference (BI) analyses were carried out for the fungi datasets. For ML analyses, tree searches were performed in IQ-tree (v.2.1.3) (Nguyen et al. 2015), based on the best-fit model GTR+F+I+I+R3 with 5000 ultrafast bootstraps (Hoang et al. 2017) in a single run. The BI search was according to the best-fit model GTR+F+I+G4, resorting to MrBayes (v.3.2.2) for BI analysis (Ronquist et al. 2012). The phylogenetic trees constructed using the ML and the BI analyses were largely congruent and strongly supported in most branches (Fig. 1). The final phylogenetic tree was visualised with its Maximum-Likelihood bootstrap proportions (ML-BS) and Bayesian posterior probability (BI-BPP) performed using FigTree v.1.4.2 and edited via Adobe Illustrator CS6.

**Table 1.** Sources of selected isolates and GenBank accession number for ITS and five genes of three genera within Polycephalomycetaceae were used in this study.

| Species name | Voucher | ITS | SSU | LSU | *TEF-1α* | *RPB1* | *RPB2* | References |
|---|---|---|---|---|---|---|---|---|
| *Cordyceps pleuricapitata* | NBRC 109979 | AB925941 | | AB925978 | | | | Unpublished |
| *Cordyceps pleuricapitata* | NBRC 109978 | AB925940 | | AB925977 | | | | Unpublished |
| *Cordyceps pleuricapitata* | NBRC 109977 | AB925939 | | AB925976 | | | | Unpublished |
| *Cordyceps pleuricapitata* | NBRC 100746 | JN943306 | JN941749 | JN941392 | KF049680 | JN992483 | KF049668 | Kepler et al. (2013) |
| *Cordyceps pleuricapitata* | NBRC 100745 | JN943304 | JN941750 | JN941391 | KF049679 | | | Kepler et al. (2013) |
| *Perennicordyceps elaphomyceticola* | MFLU 21-0262 | OQ172064 | OQ172101 | OQ172032 | OQ459718 | OQ459747 | OQ459792 | Xiao et al. (2023) |
| *Perennicordyceps cuboidea* | NBRC 100941 | JN943329 | JN941725 | JN941416 | | JN992459 | | Schoch et al. (2012) |
| *Perennicordyceps cuboidea* | NBRC 103834 | JN943330 | JN941723 | JN941418 | | JN992457 | | Schoch et al. (2012) |
| *Perennicordyceps cuboidea* | NBRC 103835 | JN943333 | JN941722 | JN941419 | | JN992456 | | Schoch et al. (2012) |
| *Perennicordyceps elaphomyceticola* | MFLU 21-0264 | OQ172067 | OQ172103 | OQ172035 | OQ459720 | OQ459749 | OQ459794 | Xiao et al. (2023) |
| *Perennicordyceps elaphomyceticola* | MFLU 21-0266 | OQ172068 | OQ172112 | OQ172036 | OQ459732 | OQ459760 | OQ459806 | Xiao et al. (2023) |
| *Perennicordyceps elaphomyceticola* | MFLU 21-0263 | OQ172065 | OQ172102 | OQ172033 | OQ459719 | OQ459748 | OQ459793 | Xiao et al. (2023) |
| *Perennicordyceps elaphomyceticola* | NTUCC 17-022 | MK840824 | | MK840813 | MK839230 | MK839221 | MK839212 | Yang et al. (2020) |
| *Perennicordyceps lutea* | KUMCC 3004 | | | OQ474910 | | | | Xiao et al. (2023) |
| *Perennicordyceps paracuboidea* | NBRC 100942 | JN943337 | JN941711 | JN941430 | | JN992445 | AB972958 | Schoch et al. (2012) |
| *Perennicordyceps prolifica* | NBRC 103839 | JN943342 | JN941706 | JN941435 | | JN992440 | | Schoch et al. (2012) |
| *Perennicordyceps prolifica* | NBRC 103838 | JN943339 | JN941707 | JN941434 | | JN992441 | | Schoch et al. (2012) |
| *Perennicordyceps prolifica* | TNS-F-18547 | KF049660 | KF049613 | KF049632 | KF049687 | KF049649 | KF049670 | Kepler et al. (2013) |

| Species name | Voucher | ITS | SSU | LSU | *TEF-1α* | *RPB1* | *RPB2* | References |
|---|---|---|---|---|---|---|---|---|
| *Perennicordyceps prolifica* | TNS-F-18481 | KF049659 | KF049612 | KF049631 | KF049686 | KF049648 | | Kepler et al. (2013) |
| *Perennicordyceps ryogamiensis* | NBRC 101751 | JN943343 | JN941703 | JN941438 | KF049688 | JN992437 | | Schoch et al. (2012) |
| *Perennicordyceps ryogamiensis* | NBRC 103837 | JN943346 | JN941702 | JN941439 | | JN992436 | | Schoch et al. (2012) |
| *Perennicordyceps ryogamiensis* | NBRC 103842 | JN943345 | JN941701 | JN941440 | | JN992435 | | Schoch et al. (2012) |
| *Pleurocordyceps parvicapitata* | MFLU 21-0270 | OQ172082 | OQ172105 | OQ172054 | OQ459722 | OQ459751 | OQ459796 | Xiao et al. (2023) |
| *Pleurocordyceps agarica* | YHHPA 1305[T] | KP276651 | KP276655 | | KP276659 | KP276663 | KP276667 | Wang et al. (2015b) |
| *Pleurocordyceps agarica* | YHCPA1307 | KP276654 | KP276658 | | KP276662 | KP276666 | KP276670 | Wang et al. (2015b) |
| *Pleurocordyceps agarica* | YHCPA 1303 | KP276653 | KP276657 | | KP276661 | KP276665 | KP276669 | Wang et al. (2015b) |
| *Pleurocordyceps aurantiaca* | MFLUCC 17-2113[T] | MG136916 | MG136904 | MG136910 | MG136875 | MG136866 | MG136870 | Xiao et al. (2019) |
| *Pleurocordyceps aurantiaca* | MFLUCC 17-2114 | MG136917 | MG136905 | MG136911 | MG136874 | | MG136871 | Xiao et al. (2019) |
| *Pleurocordyceps aurantiaca* | MFLU 17-1394 | MG136918 | MG136906 | MG136912 | MG136876 | MG136867 | MG136872 | Xiao et al. (2019) |
| *Pleurocordyceps aurantiaca* | MFLU 17-1393[T] | | MG136907 | MG136913 | MG136877 | MG136868 | MG136873 | Xiao et al. (2019) |
| *Pleurocordyceps ramosus* like | NBRC 101760 | MN586827 | MN586818 | MN586836 | MN598051 | MN598042 | MN598060 | Wang et al. (2020) |
| *Pleurocordyceps ramosus* like | NBRC 109984 | MN586828 | MN586819 | MN586837 | MN598052 | MN598043 | | Wang et al. (2020) |
| *Pleurocordyceps ramosus* like | NBRC 109985 | MN586829 | MN586820 | MN586838 | MN598053 | MN598044 | | Wang et al. (2020) |
| *Pleurocordyceps heilongtanensis* | KUMCC 3008 | OQ172091 | OQ172111 | OQ172063 | OQ459731 | OQ459759 | OQ459805 | Xiao et al. (2023) |
| *Pleurocordyceps kanzashianus* | | AB027371 | AB027325 | AB027371 | | | | Nikoh et al. (2000) |
| *Pleurocordyceps lanceolatus* | GACP 17-2004[T] | OQ172076 | OQ172110 | OQ172046 | OQ459726 | OQ459754 | OQ459800 | Xiao et al. (2023) |
| *Pleurocordyceps lanceolatus* | GACP 17-2005[T] | | OQ172109 | OQ172047 | OQ459727 | OQ459755 | OQ459801 | Xiao et al. (2023) |
| *Pleurocordyceps lianzhouensis* | HIMGD20918[T] | EU149921 | KF226245 | KF226246 | KF226248 | KF226247 | | Zhang et al. (2007) |
| *Pleurocordyceps lianzhouensis* | GIMYY9603 | EU149922 | KF226249 | KF226250 | KF226252 | KF226251 | | Zhang et al. (2007) |
| *Pleurocordyceps marginaliradians* | MFLU 17-1582[T] | MG136920 | MG136908 | MG136914 | MG136878 | MG136869 | MG271931 | Xiao et al. (2019) |
| *Pleurocordyceps marginaliradians* | MFLUCC 17-2276[T] | MG136921 | MG136909 | MG136915 | MG136879 | | MG271930 | Xiao et al. (2019) |
| *Pleurocordyceps nipponica* | BCC 1682 | KF049664 | KF049620 | KF049638 | KF049694 | | | Kepler et al. (2013) |
| *Pleurocordyceps nipponica* | BCC 18108 | KF049657 | MF416624 | MF416569 | MF416517 | MF416676 | MF416462 | Kepler et al. (2013) |
| *Pleurocordyceps nipponica* | NBRC 101407 | JN943302 | JN941752 | JN941389 | | JN992486 | | Schoch et al. (2012) |
| *Pleurocordyceps nipponica* | NBRC 101405 | JN943442 | JN941754 | JN941387 | | JN992488 | | Schoch et al. (2012) |
| *Pleurocordyceps nipponica* | BCC 2325 | KF049665 | KF049622 | KF049640 | KF049696 | KF049655 | KF049677 | Kepler et al. (2013) |
| *Pleurocordyceps nipponica* | NHJ 4268 | | KF049621 | KF049639 | KF049695 | KF049654 | KF049676 | Kepler et al. (2013) |
| *Pleurocordyceps nipponica* | BCC 1881 | | KF049618 | KF049636 | KF049692 | | KF049674 | Kepler et al. (2013) |
| *Pleurocordyceps nutansis* | GACP 19-1906 | OQ172079 | OQ172117 | OQ172049 | OQ459737 | OQ459763 | OQ459809 | Xiao et al. (2023) |
| *Pleurocordyceps nutansis* | GACP 19-1907 | OQ172087 | OQ172118 | OQ172059 | OQ459738 | OQ459764 | OQ459810 | Xiao et al. (2023) |
| *Pleurocordyceps nutansis* | GACP 19-3019[T] | OQ172086 | OQ172120 | OQ172058 | OQ459740 | OQ459766 | OQ459812 | Xiao et al. (2023) |
| *Pleurocordyceps nutansis* | MFLU 21-0275[T] | OQ172073 | OQ172119 | OQ172048 | OQ459739 | OQ459765 | OQ459811 | Xiao et al. (2023) |
| *Pleurocordyceps onorei* | BRA CR23904 | KU898843 | | | | | | Crous et al. (2017) |
| *Pleurocordyceps onorei* | BRA CR23902[T] | KU898841 | | | | | | Crous et al. (2017) |
| *Pleurocordyceps ophiocordycipiticola* | MFLUCC 22-0187 | NR185465 | | NG229093 | | | | Wei et al. (2022) |

| Species name | Voucher | ITS | SSU | LSU | *TEF-1α* | *RPB1* | *RPB2* | References |
|---|---|---|---|---|---|---|---|---|
| *Pleurocordyceps ophiocordycipiticola* | MFLU:22-0265 | OQ127364 | OQ127326 | OQ127397 | OQ186388 | OQ186435 | | Wei et al. (2022) |
| *Pleurocordyceps parvicapitata* | MFLU 21-0271[T] | OQ172083 | OQ172106 | OQ172055 | OQ459723 | OQ459752 | OQ459797 | Xiao et al. (2019) |
| *Pleurocordyceps parvicapitata* | MFLU 21-0272 | OQ172084 | OQ172099 | OQ172056 | OQ459716 | OQ459745 | OQ459790 | Xiao et al. (2023) |
| *Pleurocordyceps parvicapitata* | MFLU 21-0273 | OQ172085 | OQ172100 | OQ172057 | OQ459717 | OQ459746 | OQ459791 | Xiao et al. (2023) |
| *Pleurocordyceps phaothaiensis* | BCC84553[T] | MF959733 | | MF959737 | MF959742 | MF959745 | | Crous et al. (2017) |
| *Pleurocordyceps phaothaiensis* | BCC84552 | MF959732 | | MF959736 | MF959740 | MF959744 | | Crous et al. (2017) |
| *Pleurocordyceps phaothaiensis* | BCC84551 | MF959731 | | MF959735 | MF959739 | MF959743 | | Crous et al. (2017) |
| *Pleurocordyceps ramosopulvinata* | EFCC 5566 | | | KF049627 | KF049682 | KF049645 | | Kepler et al. (2013) |
| *Pleurocordyceps ramosopulvinata* | SU 65 | | | DQ118742 | DQ118753 | DQ127244 | | Chaverri et al. (2005) |
| *Pleurocordyceps sinensis* | ARSEF 1424 | KF049661 | KF049615 | KF049634 | KF049689 | | KF049671 | Kepler et al. (2013) |
| *Pleurocordyceps sinensis* | CN 80-2[T] | HQ832884 | HQ832887 | HQ832886 | HQ832890 | HQ832888 | HQ832889 | Wang et al. (2012) |
| *Pleurocordyceps sinensis* | HMAS 43720[T] | NR119928 | | NG042573 | | | | Wang et al. (2012) |
| *Pleurocordyceps sinensis* | MFLU 21-0269 | OQ172080 | OQ172122 | OQ172050 | OQ459742 | OQ459768 | | Xiao et al. (2023) |
| *Pleurocordyceps sinensis* | GACP 20-2305 | OQ172075 | OQ172108 | OQ172045 | OQ459725 | OQ459753 | OQ459799 | Xiao et al. (2023) |
| *Pleurocordyceps sinensis* | GACP 20-2304 | OQ172074 | OQ172107 | OQ172044 | OQ459724 | | OQ459798 | Xiao et al. (2023) |
| *Pleurocordyceps sinensis* | GZU 20-0865 | OQ172071 | OQ172096 | OQ172043 | OQ459713 | | | Xiao et al. (2023) |
| *Pleurocordyceps sinensis* | MFLU 21-0268 | OQ172070 | OQ172123 | OQ172052 | OQ459743 | | OQ459815 | Xiao et al. (2023) |
| *Pleurocordyceps sinensis* | MFLU 21-0267 | | OQ172121 | OQ172051 | | | | Xiao et al. (2023) |
| *Pleurocordyceps sinensis* | MFLU 18-0162 | MK863250 | MK863043 | MK863050 | MK860188 | | | Unpublished |
| *Pleurocordyceps* sp. | BCC 2637 | KF049663 | | KF049637 | KF049693 | | KF049675 | Kepler et al. (2013) |
| *Pleurocordyceps* sp. | JB07.08. 16_8 | KF049662 | KF049616 | KF049635 | KF049690 | KF049652 | KF049672 | Kepler et al. (2013) |
| *Pleurocordyceps* sp. | JB07.08.17_07b | | KF049617 | | KF049691 | KF049653 | KF049673 | Kepler et al. (2013) |
| *Pleurocordyceps* sp. | NBRC 109987 | | | AB925983 | | | | Unpublished |
| *Pleurocordyceps* sp. | NBRC 109988 | | | AB925984 | | | | Unpublished |
| *Pleurocordyceps* sp. | NBRC 109990 | | | AB925968 | | | | Unpublished |
| *Pleurocordyceps* sp. | NBRC 110224 | | | AB925969 | | | | Unpublished |
| *Pleurocordyceps litangensis* | YFCC 06109293 | PP410597 | PP541902 | PP410593 | PP550103 | PP697751 | | This study |
| *Pleurocordyceps litangensis* | YFCC 06109294 | PP410598 | PP541903 | PP410594 | PP550104 | PP697752 | PP550107 | This study |
| *Pleurocordyceps litangensis* | YFCC 06109295 | PP410600 | PP541905 | PP410596 | PP550106 | PP697754 | | This study |
| *Pleurocordyceps litangensis* | YFCC 06109296 | PP410599 | PP541904 | PP410595 | PP550105 | PP697753 | PP550108 | This study |
| *Pleurocordyceps* sp. | GIMCC 3.570 | | JX006097 | JX006098 | JX006100 | JX006101 | | Zhong et al. (2016) |
| *Pleurocordyceps tomentosus* | BL4 | KF049666 | KF049623 | KF049641 | KF049697 | KF049656 | KF049678 | Kepler et al. (2013) |
| *Pleurocordyceps vitellina* | KUMCC 3005 | OQ172088 | | OQ172060 | OQ459728 | OQ459756 | OQ459802 | Xiao et al. (2023) |
| *Pleurocordyceps vitellina* | KUMCC 3006 | OQ172089 | | OQ172061 | OQ459729 | OQ459757 | OQ459803 | Xiao et al. (2023) |
| *Pleurocordyceps vitellina* | KUMCC 3007 | OQ172090 | | OQ172062 | OQ459730 | OQ459758 | OQ459804 | Xiao et al. (2023) |
| *Pleurocordyceps yunnanensis* | YHCPY1005 | KF977848 | | | KF977850 | KF977852 | KF977854 | Wang et al. (2015a) |
| *Pleurocordyceps yunnanensis* | YHHPY1006[T] | KF977849 | | | KF977851 | KF977853 | KF977855 | Wang et al. (2015a) |
| *Polycephalomyces albiramus* | GACP 21-XS08[T] | OQ172092 | OQ172115 | OQ172037 | OQ459735 | OQ459761 | OQ459807 | Xiao et al. (2023) |
| *Polycephalomyces albiramus* | GACPCC 21-XS08[T] | OQ172093 | OQ172116 | OQ172038 | OQ459736 | OQ459762 | OQ459808 | Xiao et al. (2023) |
| *Polycephalomyces formosus* | NBRC 109993[T] | MN586833 | MN586824 | MN586842 | MN598057 | MN598048 | MN598064 | Wang et al. (2021) |
| *Polycephalomyces formosus* | NBRC 109994 | MN586834 | MN586825 | MN586843 | MN598058 | MN598049 | MN598065 | Wang et al. (2021) |
| *Polycephalomyces formosus* | NBRC 109995 | MN586835 | MN586826 | MN586844 | MN598059 | MN598050 | MN598066 | Wang et al. (2021) |

| Species name | Voucher | ITS | SSU | LSU | *TEF-1α* | *RPB1* | *RPB2* | References |
|---|---|---|---|---|---|---|---|---|
| *Polycephalomyces formosus* | GACP 21-WFKQ04 | OQ172095 | OQ172114 | OQ172040 | OQ459734 | | | Xiao et al. (2023) |
| *Polycephalomyces jinghongensis* | YFCC 02959283 | PP274089 | PP274093 | PP274109 | PP581803 | PP697747 | PP581819 | This study |
| *Polycephalomyces jinghongensis* | YFCC 02959284 | PP274090 | PP274094 | PP274110 | PP581804 | PP697748 | PP581820 | This study |
| *Polycephalomyces jinghongensis* | YFCC 02959285 | PP274091 | PP274095 | PP274111 | PP581805 | PP697749 | PP581821 | This study |
| *Polycephalomyces jinghongensis* | YFCC 02959286 | PP274092 | PP274096 | PP274112 | PP581806 | PP697750 | PP581822 | This study |
| *Polycephalomyces multiperitheciatae* | YFCC 06149287 | PP274102 | PP274108 | PP274118 | PP581802 | | PP581818 | This study |
| *Polycephalomyces multiperitheciatae* | YFCC 06149288 | PP274098 | PP274104 | PP274114 | PP581798 | PP697743 | PP581815 | This study |
| *Polycephalomyces multiperitheciatae* | YFCC 06149289 | PP274101 | PP274107 | PP274117 | PP581801 | PP697746 | PP581817 | This study |
| *Polycephalomyces multiperitheciatae* | YFCC 06149290 | PP274097 | PP274103 | PP274113 | PP581797 | PP697742 | PP581814 | This study |
| *Polycephalomyces multiperitheciatae* | YFCC 06149291 | PP274100 | PP274106 | PP274116 | PP581800 | PP697745 | | This study |
| *Polycephalomyces multiperitheciatae* | YFCC 06149292 | PP274099 | PP274105 | PP274115 | PP581799 | PP697744 | PP581816 | This study |
| *Polycephalomyces myrmecophilus* | YFCC 09289443 | PP410602 | PP410608 | PP410605 | PP581795 | PP697740 | PP581812 | This study |
| *Polycephalomyces myrmecophilus* | YFCC 09289444 | PP410603 | PP410609 | PP410606 | PP581796 | PP697741 | PP581813 | This study |
| *Polycephalomyces myrmecophilus* | YFCC 09289445 | PP410601 | PP410607 | PP410604 | PP581794 | PP697739 | PP581811 | This study |
| *Tolypocladium ophioglossoides* | NBRC 100998 | JN943319 | JN941735 | JN941406 | AB968602 | JN992469 | AB968563 | Ban et al. (2015) |
| *Tolypocladium ophioglossoides* | NBRC 106330 | JN943321 | JN941734 | JN941407 | AB968603 | JN992468 | AB968564 | Ban et al. (2015) |

# Results

## Phylogenetic tree

Sequences of 113 samples were used for phylogenetic analysis. *Tolypocladium ophioglossoides* (NBRC 106330) and *T. ophioglossoides* (NBRC 100998) were designated as the outgroup taxa (Xiao et al. 2023). The total length of the concatenated dataset of six genes across the 113 samples was 6384 bp, including 859 bp for ITS, 1548 bp for SSU, 930 bp for LSU, 1037 bp for *TEF-1α*, 797 bp for *RPB1* and 1213 bp for *RPB2*. The phylogenetic relationships show three major clades within the family Polycephalomycetaceae (Fig. 1), consisting of the clade *Pleurocordyceps* (16 species; BS = 100%, BPP = 1.00), the clade *Perennicordyceps* (6 species; BS = 100%, BPP = 1.00) and the clade *Polycephalomyces* (6 species; BS = 100%, BPP = 1.00). *Pleurocordyceps nutansis*, *Pleurocordyceps sinensis* (MFLU 21-0268, GZU 20-0865) are adjacent clades. Similarly, *Pleurocordyceps ramosus* like and *Pleurocordyceps yunnanensis* are contiguous branches. In addition, *Pleurocordyceps kanzashianus* is included in the clade *Pleurocordyceps nipponica*. *Cordyceps pleuricapitata* strains also formed a monophyletic clade (BS = 100%, BPP = 1.00). The four species collected and described in this work are clustered in the clade *Pleurocordyceps* (*Pl. litangensis*) and the clade *Polycephalomyces* (*Po. jinghongensis*, *Po. multiperitheciatae* and *Po. myrmecophilus*), respectively.

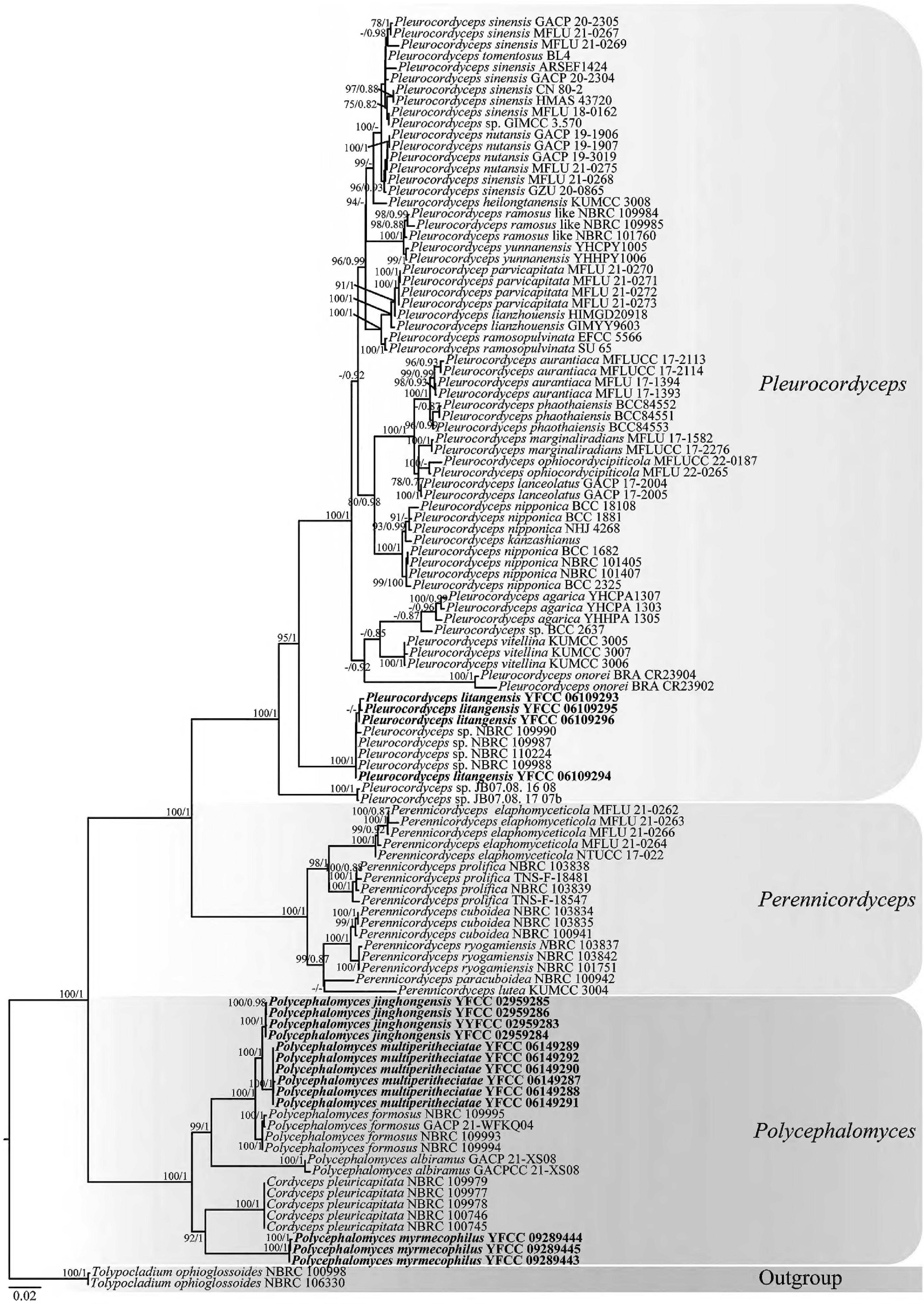


**Figure 1.** Phylogenetic tree of Polycephalomycetaceae, based on the concatenation of ITS, SSU, LSU, *TEF-1α*, *RPB1* and *RPB2* sequence data. The tree was generated from an alignment of 6,384 sites and 113 taxa. The phylogeny was inferred using the IQ-tree. The Maximum likelihood bootstrap values greater than 75% (on the left) and the Bayesian posterior probabilities over 0.75 (on the right) were indicated above the nodes. The new species were indicated in back bold font.

## Taxonomy

***Pleurocordyceps litangensis*** **Hong Yu bis, Z.H. Liu & D.X. Tang, sp. nov.**
MycoBank No: 851497
Fig. 2

**Etymology.** litangensis = Litang County, the epithet referred to the nature study trail in Litang County, the locality where the type specimen was collected.

**Diagnosis.** *Pleurocordyceps litangensis* and *Pl. sinensis* have the same host (*O. sinensis*) and β-Conidia, but the phialides (lanceolate or narrowly lageniform vs. spear point or subulate), α-conidia (Ovoid vs. Ovoid or ellipticare) are different.

**Holotype.** China, Sichuan Province, Ganzi Tibetan Autonomous Prefecture, Litang County, parasitic on *Ophiocordyceps sinensis* (Ophiocordycipitaceae), on insects buried in soil, with erect stromata, 30°43'00"N, 100°52'00"E, alt. 4750 m, 10 June 2023, Hong Yu bis (YHH 2306055).

**Sexual morph.** Undetermined.

**Asexual morph.** Synnemata arising from the stromata of *O. sinensis*, solitary or alternating; clavate or spatulate, branched and unbranched, straight or sinuous. Terminal portion of a synnemata covered by a viscous mass, khaki. Colonies on PDA growing slowly, attaining a diameter of 1.4–1.6 cm in 3 weeks at 25 °C, filiform, dark yellow and reverse dry yellow. Phialides existing in two types: α- and β-phialides. Both types of phialides often reproduce new phialides at their own apices and yield catenulate β-conidia, collarettes not flared, periclinal thickening not visible. α-phialides acropleurogenous solitary on hyphae; spear point, tapering gradually from the base to the apex, 11.2–12.8 μm long, 1.9–2.6 μm wide at the base and 0.7–0.9 μm wide at the apex. β-phialides terminal on solitary on hyphae; subulate, tapering abruptly from the base to the apex, 9.9–27.8 μm long, 1.6–2.5 μm wide at the base and 0.6–1.4 μm wide at the apex. α-conidia ovoid or elliptic and occurring on the final portion of synnemata, 3.2–6.1 × 1.8–3.9 μm; β-conidia fusiform, and produce on the surface mycelium of colony, multiple, usually in chains on a phialide, 3.5–6.1 × 1.4–2.5 μm.

**Host.** Parasitic on *Ophiocordyceps sinensis* (Ophiocordycipitaceae).

**Distribution.** China, Sichuan Province.

**Material examined.** China, Sichuan Province, Ganzi Tibetan Autonomous Prefecture, Litang County, parasitic on *Ophiocordyceps sinensis* (Ophiocordycipitaceae), on insects buried in soil, with erect stromata, 30°43'00"N, 100°52'00"E, alt. 4750 m, 10 June 2023, Tao Sun. Paratypes: YHH 2306058; other collections: YHH 2306059; Culture ex-type: YFCC 06109293; Other living cultures: YFCC 06109294, YFCC 06109295, YFCC 06109296.

**Notes.** Four strains, *Pleurocordyceps* sp. NBRC109990, *Pl*. sp. NBRC109987, *Pl*. sp. NBRC110224, *Pl*. sp. NBRC109988, were aggregated *Pl. litangensis* into one branch (Fig. 1 BS = 100%, BPP = 1.00). *Pl. litangensis* was distinct from other species of *Pleurocordyceps* by α-phialides spear point, β-phialides subulate, α-conidia ovoid or elliptic (Table 2). Thus, *Pl. litangensis* was introduced as a new species under the genus *Pleurocordyceps*.

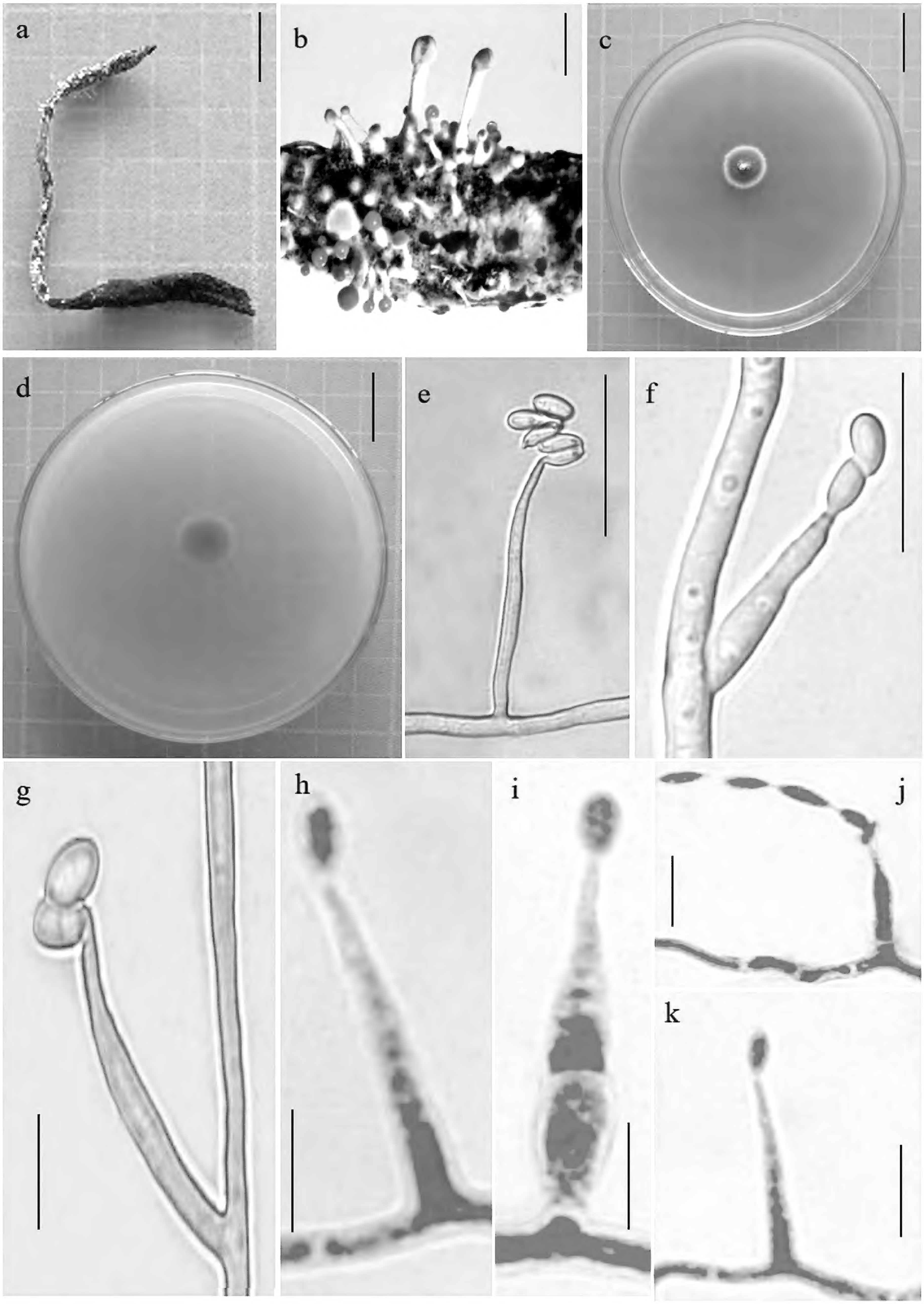


**Figure 2.** Morphological features of *Pleurocordyceps litangensis* (Holotype: YHH 2306055) **a** overview of *Pleurocordyceps litangensis* and its host **b** synnemata on the insects **c, d** colony obverse and reverse **e–h, k** α-phialides **i** α-conidia **j** β-conidia and β-phialides. Scale Bars: 2 cm (**a–d**); 20 μm (**e–j**); 10 μm (**k**); 5 μm (**g–i**).

Table 2. Morphological comparison of asexual morph species of *Pleurocordyceps*.

| Species | Host | Synnemata | Phialides | Conidia | References |
|---|---|---|---|---|---|
| *Pl. agarica* | *Ophiocordyceps* sp. or melolonthid larvae | Solitary, unbranched, agaricshaped; conidial mass pileus-like, light yellow to pale brown | α-phialides lanceolate; β-phialides narrowly lageniform or subulate | α-conidia globose to subglobose; β-conidia fusiform, catenate or clump together | Wang et al. (2015b) |
| *Pl. aurantiacus* | Coleoptera larvae or *O. barnesii* | Emerging after 30 days, solitary or not solitary, branched or unbranched, showing 1–2 radiating ring like distributions | α-phialides, narrowly lageniform. β-phialides, lanceolate or narrowly lageniform | α-conidia, globose to subglobose. β-conidia, fusiform | Xiao et al. (2018) |
| *Pl. lanceolatus* | Lepidoptera larvae | Lanceolate to corniform, solitary to crowded, stipitate, usually unbranched, rarely branched on the PDA, yellow to yellowish on the fresh specimen, covered with conidial masses, white on the PDA | α-phialides directly from hyphae, solitary, usually unbranched, subulate, at the base, tapering into a long neck; β-phialides branched into 2 or 3 phial ides, narrowly lageniform to lanceolate | α-conidia spherical, forming slimy conidial masses along the Synnemata; β-conidia fusiform | Xiao et al. (2023) |
| *Pl. marginaliradians* | Cossidae larva | Emerging after 14 days, single or branched into 2 or 3 branched, showing 1–2 radiating ring like distributions | α-phialides, elongate lageniform; β-phialides, narrow slender to narrow lageniform | α-conidia globose, catenate, one-celled, pale yellow slimy in mass. β-conidia fusiform, one-celled | Xiao et al. (2018) |
| *Pl. parvicapitata* | *Perennicordyceps elaphomyceticola* | Absent | Phialides, cylindrical at the base, tapering into a long neck | globose to subglobose | Xiao et al. (2023) |
| *Pl. sinensis* | Lepidoptera larvae or *Ophiocordyceps sinensis* | Solitary, crowded, branched or unbranched, conidial mass yellow or yellow-orange | Lanceolate or narrowly lageniform | α-conidia, ovoid; β-conidia, fusiform | Chen et al. (1984); Wang et al. (2012) |
| *Pl. vitellina* | *Ophiocordyceps nigrella* | Absent | α-phialides, hyaline, smooth, elongated lageniform, crowed, gathered in the middle of colony. β-phialides, hyaline, smooth, directly growing from hyphae, with or without metula at the base, solitary, lanceolate, ovate at the base, tapering into a short neck | α-conidia spherical, one-celled, smooth-walled. β-conidia fusiform, catenulate | Xiao et al. (2023) |
| *Pl. yunnanensis* | *Hemiptera adults* or *Ophiocordyceps nutans* | Solitary, caespitose or crowded, branched or unbranched; conidial mass white to yellow–brown | α-phialides cylindrical to subulate; β-phialides narrowly lageniform or subulate | α-conidia subglobose, ellipsoidal; β-conidia fusiform, catenate or clump together | Wang et al. (2015a) |
| *Pl. nutansis* | *Ophiocordyceps nutans* | Cylindrical, clavate, capitate, stipitate, crowded, simple, white to yellowish | Two types, both of the types observed on the same synnemata. α-phialides, gathered at the apex of the synnemata, arranged in a parallel palisade-like layer around the apex of the fertile head, hyaline, usually branched into 2–6 phialides, narrowly slender lanceolate; β-phialides , solitary, scattered along the stipe, lanceolate, ovate at the base, tapering into a long neck | α-conidia, spherical, forming slimy conidial masses on the fertile head; β-conidia fusiform, produced along stipe of the synnemata | Xiao et al. (2023) |
| *Pl. heilongtanensis* | *Ophiocordyceps* sp. | Scattered on the surface of host, cylindrical, stipitate, unbranched, white, with or without fertile head | α-phialides, hyaline, smooth, elongated lageniform, caespitose, palisade-like, crowed, gathered in the top of synnemata, mostly branched into 2–4 phialides. β-phialides hyaline, smooth, solitary, branched into 2 or 3 phial ides, with or without metula at the base, directly growing from hyphae | α-conidia, subglobose to ovoid,in yellowish slimy mass. β-conidia fusiform, one-celled | Xiao et al. (2023) |
| *Pl. lianzhouensis* | Lepidoptera larva or *Ophiocordyceps crinalis* | Unbranched or dichotomously branched, conidial mass not seen | In whorls or intercalary and terminal, terminally awl-shaped | Ellipsoidal, oblong to cylindrical | Wang et al. (2014) |
| *Pl. litangensis* | *Ophiocordyceps sinensis* | Absent | α-phialides acropleurogenous solitary on hyphae; spear point. β-phialides terminal on solitary on hyphae; subulate | α-conidia ovoid or elliptical; β-conidia fusiform | This study |

***Polycephalomyces jinghongensis*** **Hong Yu bis, Z.H. Liu & D.X. Tang, sp. nov.**
MycoBank No: 851498
Fig. 3

**Etymology.** jinghongensis = Jinghong City, the epithet referred to the nature study trail in Jinghong City, the locality where the type specimen was collected.

**Diagnosis.** *Polycephalomyces jinghongensis* are similar to that of *Po. multiperitheciatae* regarding the production of α-conidia oval, but *Po. jinghongensis* differ by synnemata caespitose, white to orange-yellow colour, producing cylindrical β-conidia, parasitic on *Ophiocordyceps* sp.

**Holotype.** China, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Jinghong City, parasitic on *Ophiocordyceps* sp. (Ophiocordycipitaceae), on insects buried in soil, with erect synnemata, 23°47'9"N, 102°51'41"E, alt. 2053 m, 25 September 2022, Hong Yu bis (YHH 2206047).

**Sexual morph.** Undetermined.

**Asexual morph.** Synnemata arising from the stromata of *Ophiocordyceps* sp., 0.8–1.6 cm long 0.1–0.3 cm thick, caespitose, unbranched or branched, white to orange-yellow colour. Colonies on PDA growing slowly, attaining a diameter of 1.3–1.7 cm in 3 weeks at 25 °C, clustered, white and reverse dry yellow. Synnemata emerging after 14 days, tufted, branched and 0.6–10 mm long, showing radiating distributions. Phialides existing in two types: α- and β-phialides. Both types of phialides often reproduce new phialides at their own apices or sides, collarettes not flared, periclinal thickening not visible. α-phialides verticillate and acropleurogenous on conidiophores and solitary on hyphae; lanceolate, tapering gradually from the base to the apex, 4.5–19.5 μm long, 1.4–2.5 μm wide at the base and 0.8–1.6 μm wide at the apex. β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; diamond-shaped; tapering abruptly from the base to the apex, 10.4–17.5 μm long, 1.1–2.7 μm wide at the base, and 0.4–1.1 μm wide at the apex. α-conidia oval or long oval shape and occurring in the conidial mass on the agar or on the final portion of synnemata, 1.1–3.4 × 0.8–1.9 μm; β-conidia columns and produced on the surface mycelium of colony, multiple, usually formed as spore balls at the phialidic apex, 2.3–3.1 × 1.2–1.3 μm.

**Host.** Parasitic on *Ophiocordyceps* sp. (Ophiocordycipitaceae).

**Distribution.** China, Yunnan Province.

**Material examined.** China, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Jinghong City, parasitic on *Ophiocordyceps* sp. (Ophiocordycipitaceae), on insects buried in soil, with erect synnemata, 23°47'9"N, 102°51'41"E, alt. 2053 m, 25 September 2022, D.X. Tang. Paratypes: YHH 2206010; other collections: YHH 2207049; YHH 2206053. Culture ex-type: YFCC 02959283; Other cultures: YFCC 02959284, YFCC 02959285, YFCC 02959286.

**Notes.** *Polycephalomyces jinghongensis* was sister to *Po. multiperitheciatae* (Fig. 1: BS = 100%, BPP = 1.00). However, *Po. multiperitheciatae* differs by 6/556 bp in ITS, 3/898 bp in SSU, 2/829 bp in LSU, 23/913 bp in *TEF-1α*, 4/679 bp in *RPB2* from *Po. jinghongensis*. *Po. jinghongensis* was distinct from other species of *Polycephalomyces* by the white to orange-yellow colour of the caespitose synnemata (Table 3). Thus, *Po. jinghongensis* was introduced as a new species under the genus *Polycephalomyces*.

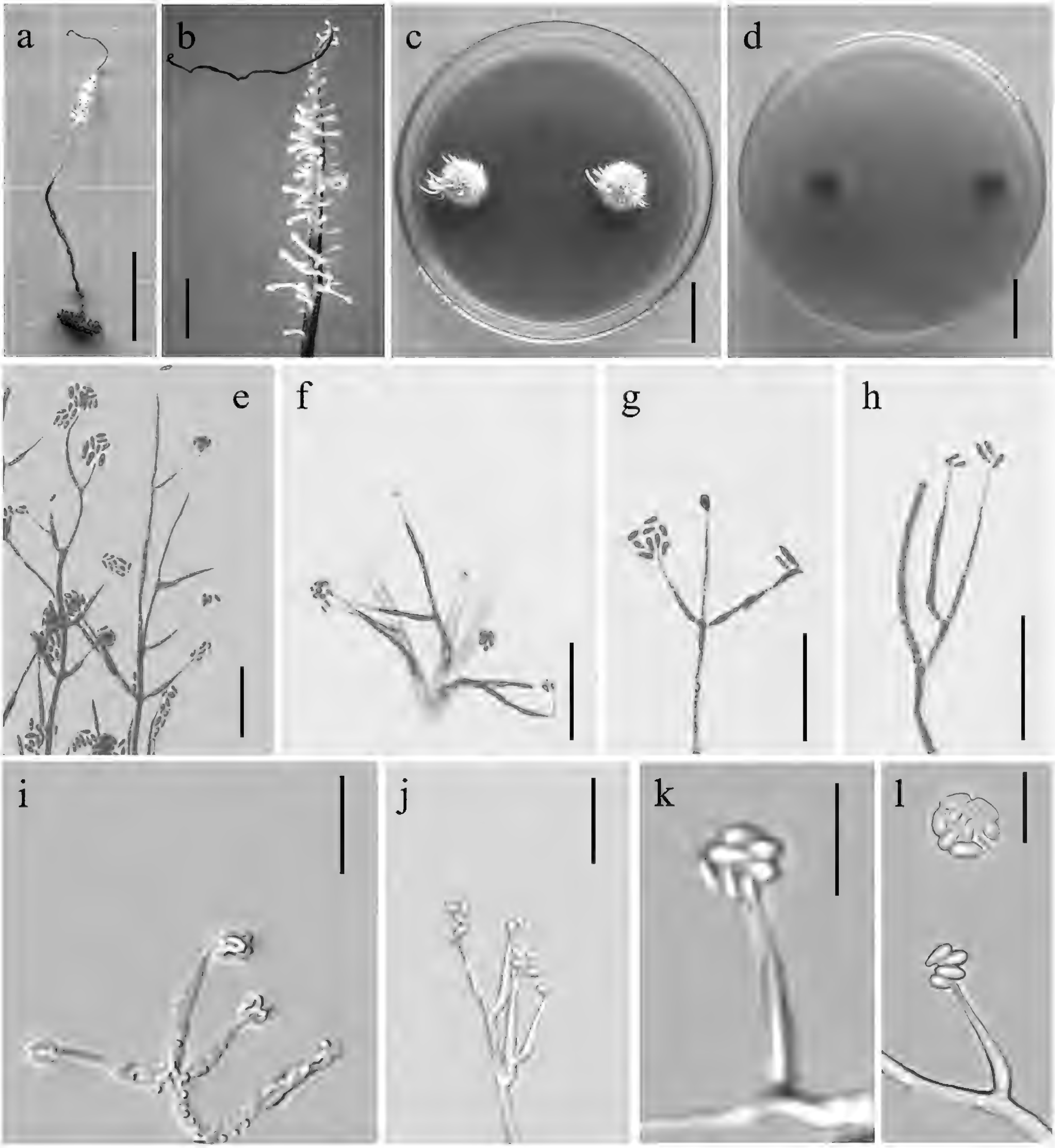


**Figure 3.** Morphological features of *Polycephalomyces jinghongensis* (Holotype: YHH 2209031) **a** overview of *Polycephalomyces jinghongensis* and its host **b** synnemata on the insect **c, d** colony obverse and reverse **e–g** β-phialides **h** β-conidia **i, k, l** α-phialides **j** α-conidia. Scale Bars: 2 cm (**a, c, d**); 0.5 cm (**b**); 20 μm (**e–h, j**); 10 μm (**i, k. l**).

***Polycephalomyces multiperitheciatae*** **Hong Yu bis, Z.H. Liu & D.X. Tang, sp. nov.**
MycoBank No: 851499
Fig. 4

**Etymology.** The species name referred to the host species, *Ophiocordyceps multiperitheciata*.

**Diagnosis.** *Polycephalomyces multiperitheciatae* are similar to that of *Po. jinghongensis* regarding the production of α-conidia oval, but *Po. jinghongensis*

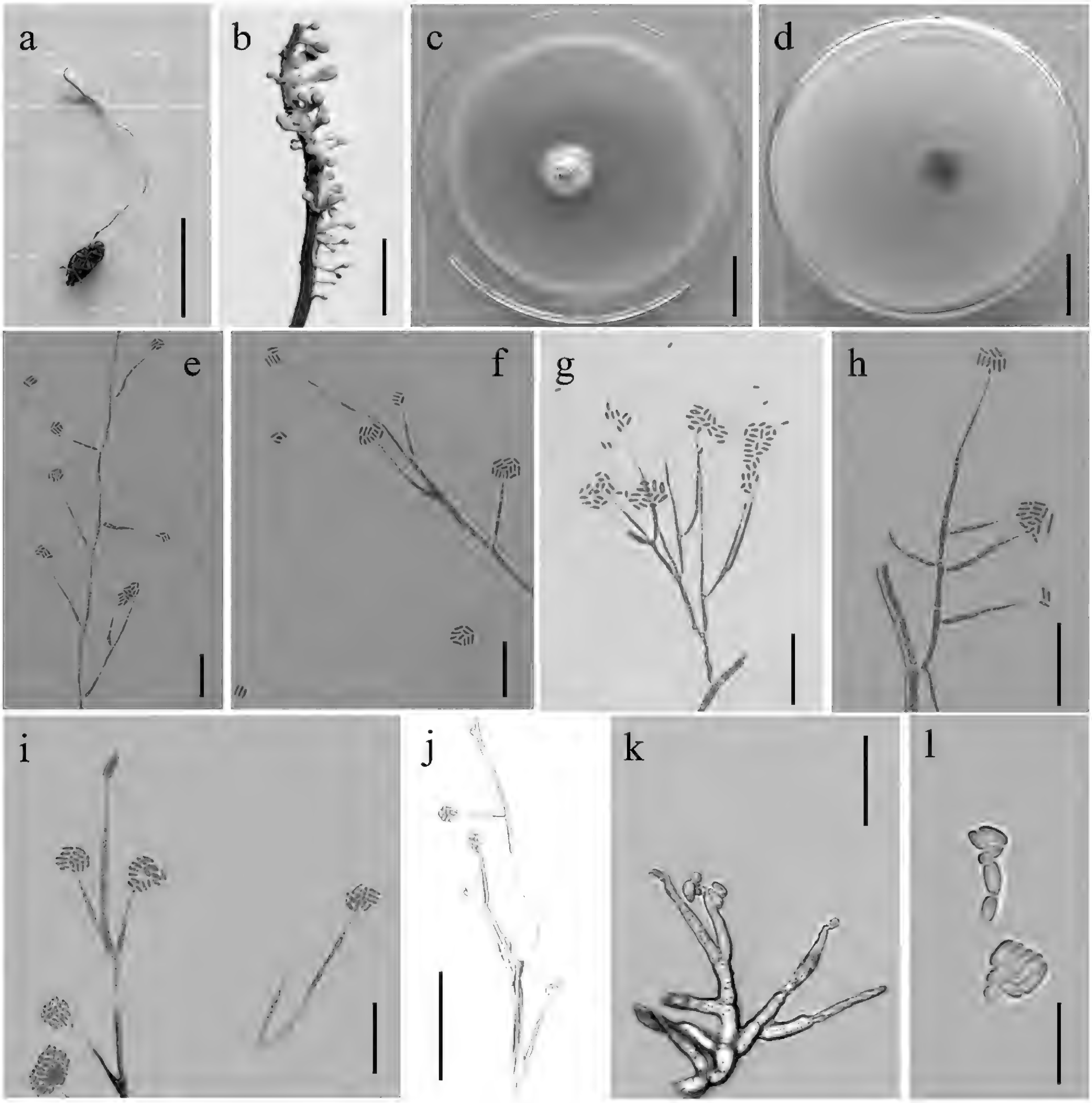


**Figure 4.** Morphological features of *Polycephalomyces multiperitheciatae* (Holotype: YHH 2206047) **a** overview of *Polycephalomyces multiperitheciatae* and its host **b** Synnemata on the insect **c, d** colony obverse and reverse **g, j** α-phialides **e, f, h, i** β-phialides **k** α-conidia **l** β-conidia. Scale Bars: 2 cm (**a, c, d**); 0.6 cm (**b**); 20 μm (**e–i, k**); 50 μm (**j**); 10 μm (**l**).

differ by being parasitic on *O. multiperitheciata*, synnemata clustered, white, β-conidia, linear.

**Holotype.** China, Yunnan Province, Honghe Hani and Yi Autonomous Prefecture, Yuanyang County, parasitic on *Ophiocordyceps multiperitheciata* (Ophiocordycipitaceae), on insects buried in soil, with erect stromata, 22°1'51"N, 100°52'42"E, alt. 703 m, 25 September 2022, Hong Yu bis (YHH 2206031).

**Sexual morph.** Undetermined.

**Asexual morph.** Synnemata arising from the stromata of *Ophiocordyceps multiperitheciata*, 0.8–1.8 cm long 0.2–0.5 cm thick, clustered, white to pale yellow, numerous, branched, with fertile head. Colonies on PDA growing slowly,

attaining a diameter of 1.8–2.1 cm in 3 weeks at 25 °C, clustered, white and reverse dry yellow. Synnemata emerging after 15 days, solitary, branched and 0.8–2.1 cm long, showing radiating distributions. Phialides existing in two types: α- and β-phialides. Both types of phialides often reproduce new conidia at their own apices or sides, collarettes not flared, periclinal thickening not visible. α-phialides verticillate and acropleurogenous on conidiophores and solitary on hyphae; spear point, tapering gradually from the base to the apex, 10.5–18.7 μm long, 1.1–1.9 μm wide at the base and 0.4–0.6 μm wide at the apex. β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; subulate, tapering abruptly from the base to the apex, 11.3–28.8 μm long, 1.2–2.5 μm wide at the base and 0.5–1.1 μm wide at the apex. α-conidia,oval and occurring in the conidial mass on the agar or on the final portion of synnemata, 0.6–1.1 × 0.3–0.6 μm; β-conidia, linear and produced on the surface mycelium of colony, multiple, usually formed as spore balls at the phialidic apex, 0.8–1.3 × 0.3–0.7 μm.

**Host.** Parasitic on *Ophiocordyceps multiperitheciata* (Ophiocordycipitaceae).

**Distribution.** China, Yunnan Province.

**Material examined.** China, Yunnan Province, Honghe Hani and Yi Autonomous Prefecture, Yuanyang County, parasitic on *Ophiocordyceps multiperitheciata* (Ophiocordycipitaceae), on insects buried in soil, with erect stromata, 22°1'51"N, 100°52'42"E, alt. 703 m, 25 September 2022, D.X. Tang. Paratypes: YHH 2209032; other collections: YHH 2209033; YHH 2209034. Culture ex-type: YFCC 06149287; Other cultures: YFCC 06149288, YFCC 06149289, YFCC 06149290, YFCC 06149291, YFCC 06149292.

**Notes.** *Polycephalomyces multiperitheciatae* is sister to *Po. jinghongensis* (Fig. 1: BS = 100%, BPP = 1.00). *Po. multiperitheciatae* is distinct from other species of *Polycephalomyces*, parasitising *Ophiocordyceps multiperitheciata* synnemata clustered, with fertile head, β-conidia, linear (Table 3). Thus, *Po. multiperitheciatae* was introduced as a new species under the genus *Polycephalomyces*.

### ***Polycephalomyces myrmecophilus*** **Hong Yu bis, Z.H. Liu & D.X. Tang, sp. nov.**

MycoBank No: 851500

Fig. 5

**Etymology.** myrmecophilus = myrmecophilous, the epithet referred to the species parasitising myrmecophilous *Ophiocordyceps* species.

**Diagnosis.** *Polycephalomyces myrmecophilus* are similar to that of *Po. ramosus* regarding the production of two types of conidia, but *Po. myrmecophilus* differ by α-conidia round or ovoid, β-conidia elliptical.

**Holotype.** China, Yunnan Province, Pu'er City, The Sun River National Forest Park, parasitic on *Ophiocordyceps acroasca* (Ophiocordycipitaceae), on insects underside of leaves, with erect stromata, 30°34'34"N, 101°6'24"E, alt. 1095 m, 28 September 2020, Hong Yu bis (YHH 2009001);

**Sexual morph.** Undetermined.

**Asexual morph.** Synnemata arising from the *Ophiocordyceps acroasca* or *Colobopsis* sp. corpses, tomentose, white. Colonies on PDA growing slowly, attaining a diameter of 1.7–2.1 cm in 3 weeks at 25 °C, villous, cinerous, and reverse black yellow. Phialides existing in two types: α- and β-phialides. Both types of phialides

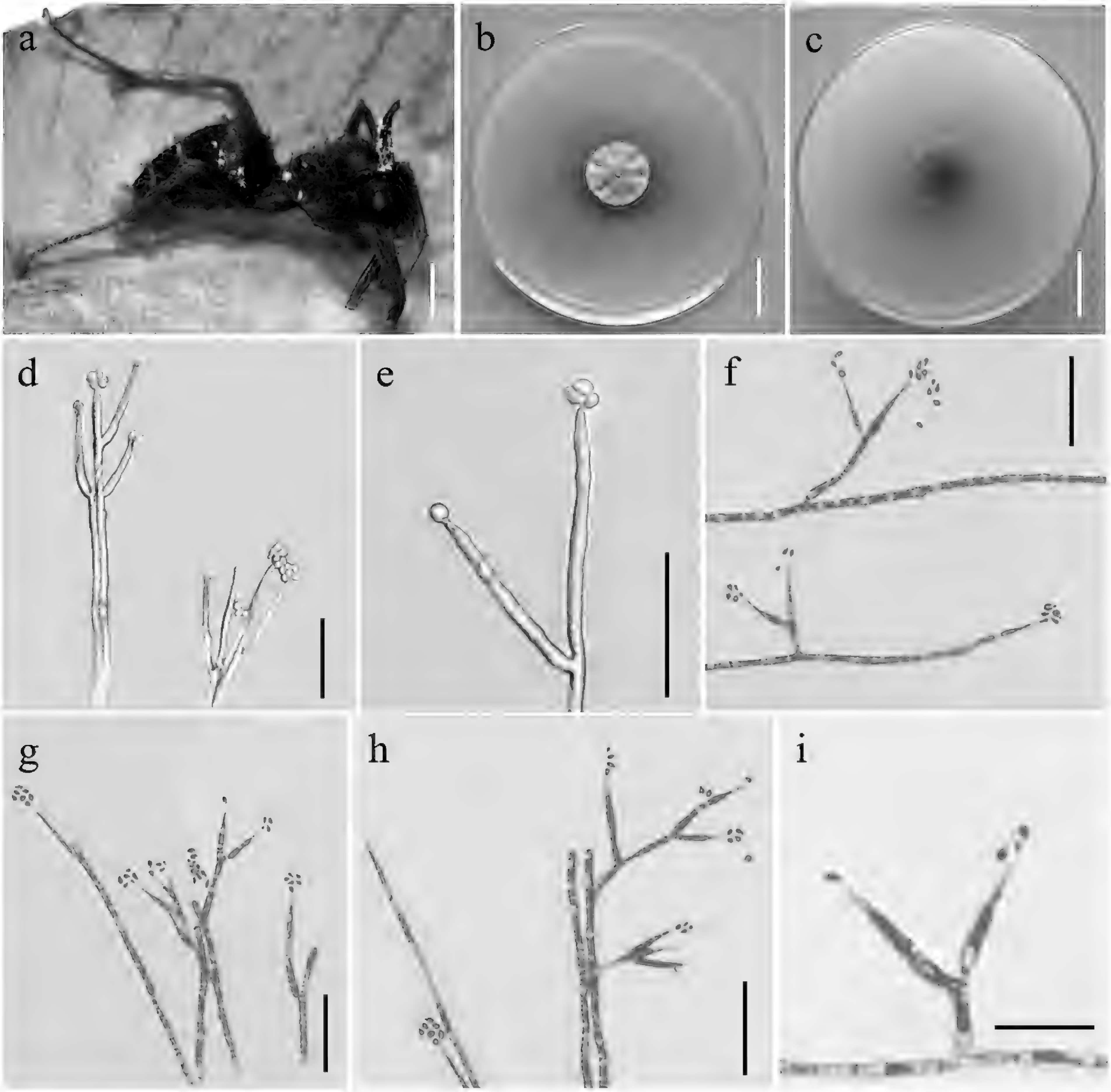


**Figure 5.** Morphological features of *Polycephalomyces myrmecophilus* (Holotype: YHH 2009001) **a** overview of *Polycephalomyces myrmecophilus* and its host **b, c** colony obverse and reverse **d** α-phialides **f–h** β-phialides **e** α-conidia **i** β-conidia. Scale Bars: 2 cm (**a–c**); 20 μm (**d–h**); 10 μm (**i**).

often reproduce new phialides at their own apices, collarettes not flared, periclinal thickening not visible. α-phialides verticillate and acropleurogenous on conidiophores and solitary on hyphae; lanceolate, tapering gradually from the base to the apex, 6.1–14.5 μm long, 1.4–2.3 μm wide at the base and 0.8–1.8 μm wide at the apex. β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; sickle-shaped, tapering abruptly from the base to the apex, 9.8–17.6 μm long, 0.9–1.6 μm wide at the base and 0.4–1.1 μm wide at the apex. α-conidia round or ovoid, and occurring in the conidial mass on the agar or on the final portion of synnemata, 0.4–0.9 × 0.3–0.9 μm; β-conidia elliptical and produced on the surface mycelium of colony, single or multiple, usually in the form of spore balls at the phialidic apex, 0.6–1.3 × 0.3–0.8 μm.

**Table 3.** Morphology of asexual morph species of the genus *Polycephalomyces*.

| Species | Host | Synnemata | Phialides | Conidia | References |
|---|---|---|---|---|---|
| *Po. albiramus* | *Gryllotalpa* sp. (Orthoptera, Gryllotalpidae) | Stipitate, gathered, branched, white to pale yellow, numerous, cylindrical and tapering at the apex, without fertile head | Phialides narrowly subulate, awl-shaped | Conidia cylindrical to obovoid or subglobose | Xiao et al. (2023) |
| *Po. baltica* | Nymph or short-winged female bark louse (Psocoptera: Troctopsocidae) | Synnemata, simple, roundish | Phialides, light colored, micronematous, flask-shaped | Conidia globose, catenulate | Poinar and Vega (2020) |
| *Po. cylindrosporus* | Coleoptera, Formicidae and Pentatomidae | Synnemata cylindrical to capitate, stipitate, slender, branched | Phialides on verticils and/or acropleurogenously forming loosely arranged flared hymenia | Conidia one-type, cylindrical to bacilliform | Matočec et al. (2014) |
| *Po. ditmarii* | *Paravespula vulgaris* (Wasp) | Synnemata 2 to 3 distinct branches, yellowish to white, darkening at the base; surmounted by a small subsurface capitulum, dotted with numerous small blisters of orange-yellow colour | Phialides elongate, cylindrical, attenuating at the apex | globose to subglobose | Van Vooren and Audibert (2005) |
| *Po. formosus* (Type) | Coleoptera larvae or *Ophiocordyceps barnesii* | Synnemata 2 long, gathered, branched, with cylindrical stipe, with fertile head, spherical, white | cylindrical, tapering gradually | Conidia one-type, ellipsoid or ovoid | Kobayasi (1941) |
| | In culture (PDA) | Synnemata 2–3 branches,arising as several radiating rings on the colony | Phialides terminal parts of Synnemata, cylindrical to subulate at the base; | Conidia of one type, one-celled, smooth-walled, ellipsoid to ovoid, arising in a conidial mass on the agar or on the terminal portions of synnemata | Wang et al. (2021) |
| | In slide culture | | Phialides monothetic and solitary or acropleurogenous in the whorls of 1–4, narrowly lageniform or subulate | Conidia obovoid to oblong ellipsoidal or cylindrical, forming irregular spore balls near the apex of phialides | Wang et al. (2021) |
| *Po. ramosus* | Lepidoptera larvae or *Hirsutella guignardii* | Synnemata solitary, crowded or caespitose, unbranched or branched, conidial mass yellow to orange-yellow | α-phialides cylindrical to narrowly lageniform; β-phialides narrowly lageniform or subulate | α-conidia, ovoid; β-conidia, fusiform | Seifert (1985); Bischof et al. (2003) |
| *Po. paludosus* | Lepidoptera larva | Capitate, cinnamon brown, branched, the branches at right angles | Subulate, phialides occurring scattered on the branches below the heads, ventricose, occasionally stellate above | Conidia produced singly, hyaline, obovoid, covered by agglutinated mucus | Mains (1948) |
| *Po. tomentosus* | Myxomycetes | Fructification a synnemata | | Conidia three-type, globose or ellipsoidal or cylindrical | Seifert (1985) |
| *Po. jinghongensis* | *Ophiocordyceps* sp. (Ophiocordycipitaceae) | Synnemata caespitose, unbranched or branched, white to orange-yellow colour | α-phialides verticillate and acropleurogenous on conidiophores,and solitary on hyphae; lanceolate. β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; diamond-shaped. | α-conidia oval or long oval shape, β-conidia cylindrical | This study |
| *Po. multiperitheciatae* | *Ophiocordyceps multiperitheciata* | Synnemata white to pale yellow, numerous, branched, with fertile head | α-phialides verticillate and acropleurogenous on conidiophores, and solitary on hyphae; spear point. β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; subulate. | α-conidia oval β-conidia linear | This study |
| *Po. myrmecophilus* | *Ophiocordyceps acroasca* and *Ophiocordyceps* sp. | Absent | α-phialides verticillate and acropleurogenous on conidiophores, and solitary on hyphae; lanceolate, β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; sickle shape. | α-conidia round or ovoid; β-conidia, elliptical | This study |

**Host.** Parasitic on *Ophiocordyceps acroasca* and *Ophiocordyceps* sp.

**Distribution.** China, Yunnan Province.

**Material examined.** China, Yunnan Province, Pu'er City, The Sun River National Forest Park, parasitic on *Ophiocordyceps acroasca* (Ophiocordycipitaceae), on insects underside of leaves, with erect stromata, 30°34'34"N, 101°6'24"E, alt. 1095 m, 28 September 2020, D.X. Tang. Paratype: YHH 2006020. Culture ex-type: YFCC 09289443; Other cultures: YFCC 09289444.

**Notes.** *Polycephalomyces myrmecophilus* was sister to *Cordyceps pleuricapitata* (Fig.1: BS = 100%, BPP = 1.00). *Po. myrmecophilus* was distinct from other species of *Polycephalomyces*, being parasitic on *Ophiocordyceps acroasca* and *Ophiocordyceps* sp. and producing β-phialides sickle-shaped, α-conidia round or ovoid, β-conidia elliptical (Table 3). Thus, *Po. myrmecophilus* was introduced as a new species under the genus of *Polycephalomyces*.

## Discussion

Our taxonomic investigations revealed four new species of the family Polycephalomycetaceae, *Pl. litangensis*, *Po. jinghongensis*, *Po. multiperitheciatae* and *Po. myrmecophilus*. Morphological observations suggested that four species have sufficient morphological differences to justify their segregation into four species. A new species, *Pl. litangensis*, was described in the genus *Pleurocordyceps*. *Pleurocordyceps litangensis* was similar to *Pl. agaricus*, *Pl. aurantiacus*, *Pl. lanceolatus*, *Pl. marginaliradians*, *Pl. sinensis*, *Pl. vitellina*, *Pl. yunnanensis*, *Pl. nutansis* and *Pl. heilongtanensis*, by producing two types of conidia, while *Pl. Parvicapitata* and *Pl. lianzhouensis* had only one type of conidia. *Pl. litangensis* was distinct from other species of *Pleurocordyceps*, with having α-phialides spear point, β-phialides subulate, α-conidia ovoid or elliptic. Moreover, *Pl. litangensis* and *Pl. sinensis* both had the same host (*O. sinensis*) and β-Conidia, but their phialides, α-conidia size and shape were different (Table 2). Herein, we described three new species, namely, *Po. jinghongensis*, *Po. multiperitheciatae* and *Po. myrmecophilus*, enriching the species diversity in the genus *Polycephalomyces*. Six additional species are included in this genus (Table 1): *Polycephalomyces baltica* (Poinar and Vega 2020), *Po. cylindrosporus* (Matočec et al. 2014), *Po. ditmarii* (Van Vooren and Audibert, 2005), *Po. paludosus* (Mains 1948), *Po. ramosus* (Seifert 1985; Bischof et al. 2003) and *Po. tomentosus* (Seifert 1985). These species either lacked molecular data or their updated strain descriptions did not match those of the protologue (Wang et al. 2021). These three new species were similar to *Po. ramosus*, producing two types of conidia, while *Po. baltica*, *Po. cylindrosporus*, *Po. ditmarii*, *Po. paludosus* and *Po. albiramus* (Xiao et al. 2023) had only one type of conidia. *Po. jinghongensis* was distinct from *Po. ramosus*, being parasitic on *Ophiocordyceps* sp. producing longer α-conidia oval or long oval shape and β-conidia columns. *Po. multiperitheciatae* differed from *Po. ramosus*, being parasitic on *O. multiperitheciata*, having synnemata with fertile head and β-conidia linear. *Po. myrmecophilus* was distinguished from *Po. ramosus*, being parasitic on the fungus *O. acroasca*, producing synnemata, α-conidia round or ovoid, and β-conidia elliptical, without producing synnemata from the colonies, whereas *Po. ramosus* was parasitic on Lepidoptera larvae or *Hirsutella guignardii*, with α-conidia ovoid and β-conidia fusiform (Table 3).

Some species of the family Polycephalomycetaceae have been reported from more than one host, indicating their non-host specific nature (Bischof et

al. 2003; Wang et al. 2012, 2015a, b; Matočec et al. 2014; Crous et al. 2017; Xiao et al. 2018). *Pl. lianzhouensis* (Wang et al. 2014) was found to parasitise insects along with the species of the genus *Ophiocordyceps*. The field investigation and studies showed that *Pl. litangensis* also parasitised *O. sinensis*, a phenomenon known as hyperparasitism. Most species of the genus *Polycephalomyces* parasitise insects in the orders Coleoptera and Hemiptera, and we have already discovered that *Po. jinghongensis*, *Po. multiperitheciatae* and *Po. myrmecophilus* are hyperparasitic on the species of *Ophiocordyceps*, expanding the diversity of hosts in *Polycephalomyces*. In subsequent studies, we should delve deeper into the ecological habits and hyperparasitic phenomena of the family Polycephalomycetaceae, explore the evolutionary relationship between hyperparasitic species and entomophytic fungi and promote their development and utilisation.

Xiao et al. (2023) introduced *Pl. nutansis* as a new species under the genus *Pleurocordyceps*. However, *Pl. sinensis* and *Pl. nutansis* were found to be grouped together in the phylogenetic tree, which may be the reason and they are sister taxa to each other. Similarly, molecular phylogenetic analysis has shown that *Pl. nipponica* and *Pl. kanzashianus* are clustered together. Nevertheless, Wang et al. (2021) pointed out that they were distinct species, based on their sexual morphology characteristics. In addition, Wang et al. (2021) noted the description of the spore type of *Pl. lianzhouensis* was not clear and future research should strengthen the observation of its asexual morphology to determine its more accurate classification position. *Cordyceps pleuricapitata* has formed a monophyletic branch in the genus *Polycephalomyces*. Xiao et al. (2023) noted the paratype of *C. pleuricapitata* lacks molecular data and the two strains (NBRC 100745, NBRC 100746) named *C. pleuricapitata* for which there are molecular data lack morphological information. Hence, it was not possible to clarify the precise position of *C. pleuricapitata* and its classification at this time. These classifications issues require further research. Phylogeny based on our concatenated data also supported that our four new species belonged to the family Polycephalomycetaceae and were distinct from each other (Fig. 1). Four strains, namely, *Pleurocordyceps* sp. NBRC109990, *Pleurocordyceps* sp. NBRC109987, *Pleurocordyceps* sp. NBRC110224 and *Pleurocordyceps* sp. NBRC109988 and *Pl. litangensis* were aggregated into one branch. However, the four strains had only LSU sequences in the NCBI database and were classified as undefined species in *Pleurocordyceps* incertae sedis. Future research will require additional morphological and phylogenetic work to clarify their taxonomic status.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.

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## Author contributions

Zuoheng Liu: Mainly responsible for article conception, writing and editing and also mainly responsible for species identification (contributed equally to this work); Dexiang Tang: Mainly responsible for article conception writing and editing, morphological analysis and phylogenetic analysis(contributed equally to this work); Yingling Lu: Mainly responsible for article conception, and also responsible for experimental guidance and design; Responsible for the language polishing and format modification.Juye Zhu: Collecting the information of specimens and GenBank entry number required for research. Lijun Luo; Tao Sun: Responsible for picture editing and processing. Hong Yu: Investigation, responsible for the review and modification of the article, and conducting project administration and supervision.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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